PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	21.04.2005
Applicant's or agent's file reference: PN02116-PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NO 03/00443	29.12.2003	30.12.2002

Applicant
AMERSHAM HEALTH AS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

DUE DATE:	—
FORMALITIES:	MN
PAT. OFF:	LHT
ON DB:	26-Apr-2005
CASE NO:	PN02116PC

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PN02116-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NO 03/00443	International filing date *(day/month/year)* 29.12.2003	Priority date *(day/month/year)* 30.12.2002
International Patent Classification (IPC) or both national classification and IPC C07K2/00		
Applicant AMERSHAM HEALTH AS et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.07.2004	21.04.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Jenn, T Telephone No. +49 89 2399-7348

JC20 Rec'd PCT/PTO 22 JUN 2005

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NO 03/00443

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-3, 5-17	as published
4	filed with telefax on 04.04.2005

Sequence listings part of the description, Pages

1-3	as published

Claims, Numbers

1-11	filed with telefax on 04.04.2005

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 11

   because:

   ☒ the said international application, or the said claims Nos. 11 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations

see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET International application No. PCT/NO 03/00443

Re Item I

Basis of the report

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Reference is made to the following documents:

D3: WO 01/52875 A (LUDWIG INST CANCER RES) 26 July 2001;

D4: WO 99/40947 A (ESHIMA DENNIS et al.) 19 August 1999.

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The application discloses (the references in parentheses applying to this document) a targetable diagnostic and/or therapeutically active agent of formula (III) V-L-Z, wherein L represents a bond, a spacer or a linker, Z is an antineoplastic agent, a reporter moiety or a group that optionally can carry an imaging moiety M and V is a peptide of formula (I) Z^1-R-X^2-X^3-I-X^5-X^6-X^7-X^8-X^9-Z^2-Y^1, wherein X^2 is selected from V, L, I and Y; X^3 is selected from R, K, Y, I, N; X^5 is D or N; X^6 is G, N or Q; X^7 is A, M, Q, R, E or V; X^8 is P, G, S or R; X^9 is A, M, Q, R, G or V, Z_1 is absent or C or Hcy or a residue capable of forming a disulphide or a thioether bond; Z^2 is absent or C or Hcy or a residue capable of forming a disulphide bond; Y^1 is absent or represents 1-10 amino acids (claims 1-7). The application discloses as well a peptide comprising the amino acid sequence of formula (II) Z^1-R-V-X^3-I-D-G-X^7-P-X^9-Z^2-Y^1, wherein X^3 is selected from R, K, Y, I, N; X^7 is A, M, Q, R, E or V; X^9 is A, M, Q, R, G or V, Ż is absent or C or Hcy or a residue capable of forming a disulphide or a thioether bond; Z^2 is absent or C or Hcy or a residue capable of forming a disulphide bond; Y^1 is absent or represents 1-10 amino acids (claim 8); or a peptide comprising the amino acid sequences as disclosed in claim 9 (claim 9); a pharmaceutical composition comprising a compound of formula (III) (claim 10); and a method of generating enhanced images of a human or animal body previously administered with a contrast agent composition comprising a compound of formula (III) (claim 11).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The method as claimed in **claim 11** relates to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT (diagnostic method carried out on the living human or animal body). Consequently, no opinion will be formulated on the industrial applicability of the subject-matter of this claim (Article 34(4)(a)(i) PCT, see also the PCT-guidelines IV-2.4.(d) and IV-2.5); an opinion on novelty and inventive step will be given for the alleged effects of a compound of claim 1 in the method of claim 11.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 **Claims 1-7 and 10-11:**

1.1 The document **D4** is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (the references in parentheses applying to this document) a compound for imaging and treatment of angiogenesis, which is of formula (I) A-$(B)_n$-C, wherein A is a chelator moiety capable of complexing a radionuclide metal or a moiety capable of binding to a halogen; B is a spacer group; C is an angiogenesis targeting molecule; and n is 0 or 1 (Abstract).

1.2 The subject-matter of claim 1 therefore **differs** from this known compound in that it comprises the amino acid sequence of formula (I) of the application.

1.3 The subject-matter of claim 1 can therefore be considered **new**.

1.4 The **problem** to be solved by the present invention may therefore be regarded as to provide alternative compounds for imaging.

1.5 The **solution** to this problem proposed in claim 1 of the present application is considered

as involving an **inventive** step (Article 33(3) PCT), because a compound of formula (III) is not suggested by the available prior art documents.

1.6 A pharmaceutical composition comprising the new and inventive compound of formula (III), and the use of said compound in a method of generating enhanced images of a human or animal body can also be considered new and inventive.

1.7 Therefore, the subject-matter of **claims 1-7, 10 and 11 complies** with the requirements of Article 33(2) and 33(3) PCT.

2 **Claims 8 and 9**:

2.1 The document **D3** is regarded as being the closest prior art to the subject-matter of claims 8 or 9, and discloses a series of monomeric monocyclic peptide inhibitors and dimeric bicyclic peptide inhibitors based on exposed loop fragments of the growth factor VEGF-D, VEGF-C or VEGF, methods of making them as well as pharmaceutical compositions containing them and methods (for imaging) utilizing them (Abstract, claims 1, 48, 49, 66 and 69). None of said peptides comprises the amino acid sequence of formula (I) of the application.

2.2 The subject-matter of claims 8 and 9 therefore **differs** from this known compound in that it claims a peptide comprising the amino acid sequence of formula (II) or a peptide comprising the amino acid sequence SEQ ID No: 1-10 (all peptides comprising the amino acid sequence of formula (I) of the application).

2.3 The subject-matter of claims 8 and 9 can therefore be considered **new**.

2.4 The **problem** to be solved by the present invention may therefore be regarded as to provide alternative peptides for imaging.

2.5 The **solution** to this problem proposed in claims 8 and/or 9 of the present application is considered as involving an inventive step (Article 33(3) PCT), because a peptide comprising the amino acid sequence of formula (II) or a peptide comprising the amino acid sequence SEQ ID No: 1-10 are not suggested by the available prior art documents.

2.6 Therefore, the subject-matter of **claims 8 and 9 complies** with the requirements of Article 33(2) and 33(3) PCT.

3 An agent of formula (III) according to claim 1 has an application for preparing a pharmaceutical composition; and a peptide according to claim 9 or 10 is comprised in an agent of formula (III). Therefore, the subject-matter of claims 1-10 complies with the requirements of Article 33(4) PCT.

4 **Certain observations on the international application**

4.1 The embodiments of the invention described on page 15 (the whole Example 2) do not fall within the scope of the claims (the peptide according to Example 2 is not of formula (I), as it comprises a Lys at the position for Z^1, and a Pro at the position for X^6, which do not enter in the definition of Z^1 and X^6 given in claim 1).
This inconsistency between the claims and the description leads to doubt concerning the matter for which protection is sought, thereby rendering the claims unclear (Article 6 PCT).

4.2 The features of claim 4 are not referred to in the description. Claim 4 is therefore not supported by the description as required by Article 6 PCT.

4.3 Attention is drawn to the following: The use of the expression "*incorporated by reference*" (page 6, line 8; page 8, line 3; and page 9, line 15) is not allowed in some designated Contracting States.

4.4 There is a spelling mistake in claim 5: "An agent as claimed in claim 5" for "An agent as claimed in claim 4".

Claims

1. A targetable therapeutically active and/or diagnostic agent of formula (III)

$$V-L-Z \qquad (III)$$

wherein the vector V is a peptide comprising the amino acid sequence of formula (I)

$$Z^1\text{-Arg-}X^2\text{-}X^3\text{-Ile-}X^5\text{-}X^6\text{-}X^7\text{-}X^8\text{-}X^9\text{-}Z^2\text{-}Y^1 \qquad (I)$$

or formula (II)

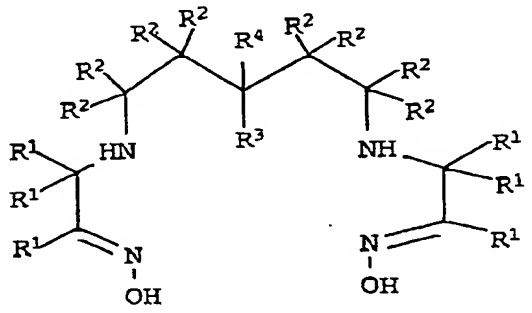
$$Z^1\text{-Arg-Val(Arg/Lys)Ile-Asp-Gly-}X^7\text{-Pro-}X^9\text{-}Z^2\text{-}Y^1 \qquad (II)$$

wherein
X^2 is an amino acid selected from the group Val, Leu, Ile and Tyr
X^3 is an amino acid selected from the group Arg, Lys, Tyr, Ile and Asn
X^5 is an amino acid selected from the group Asp and Asn
X^6 is an amino acid selected from the group Gly, Asn and Gln
X^7 is an amino acid selected from the group Ala, Met, Gln, Arg, Glu and Val.
X^8 is an amino acid selected from the group Pro, Gly, Ser and Arg
X^9 is an amino acid selected from the group Ala, Met, Gln, Arg, Gly and Val
Z^1 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue, or a residue capable of forming a thioether preferably the residue is Q-C(=O) wherein Q represents $-(CH_2)n$ or $-(CH_2)_n\text{-}C_6H_4$ where n represents a positive integer 1 to 10 or is absent and
Z^2 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue or is absent
Y^1 represents 1-10 amino acids or is absent
L represents a bond, a spacer or a linker and
Z represents an antineoplastic agent, a reporter or a group that optionally can carry an imaging moiety M.

2. A targetable therapeutically active and/or diagnostic agent according to claim 1 wherein the vector V is a peptide comprising the amino acid sequence
Cys-Arg-Val-Arg-Ile-Asp-Gly-Ala-Pro-Ala-Cys, (SEQ ID NO 1),
Cys-Arg-Val-Arg-Ile-Asp-Asn-Met-Pro-Met-Cys, (SEQ ID NO 2),
Cys-Arg-Val-Arg-Ile-Asn-Gly-Gln-Pro-Gln-Cys, (SEQ ID NO 3),
Cys-Arg-Val-Lys-Ile-Asp-Gly-Arg-Pro-Met-Cys, (SEQ ID NO 4),
Cys-Arg-Leu-Lys-Ile-Asp-Gly-Met-Pro-Arg-Cys, (SEQ ID NO 5),
Cys-Arg-Ile-Lys-Ile-Asp-Gly-Glu-Gly-Gln-Cys, (SEQ ID NO 6),
Cys-Arg-Val-Tyr-Ile-Asp-Gly-Val-Ser-Val-Cys, (SEQ ID NO 7),
Cys-Arg-Val-Ile-Ile-Asp-Gly-Arg-Arg-Met-Cys, (SEQ ID NO 8),

Cys-Arg-Tyr-Asn-Ile-Asp-Gly-Arg-Pro-Gln-Cys, (SEQ ID NO 9) or
Cys-Arg-Ile-Arg-Ile-Asp-Gln-Arg-Pro-Ala-Cys. (SEQ ID NO 10).

3. An agent according to any of the previous claims 1 and 2 where Z is a chelating agent of formula IV



(IV)

where:
each R^1, R^2, R^3 and R^4 is independently an R group:
each R group is independently H or C_{1-10} alkyl, C_{3-10} alkylaryl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} alkylamine, C_{1-10} fluoroalkyl, or 2 or more R groups, together with the atoms to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring.

4. An agent as claimed in claim in any of the previous claims 1 to 3 wherein Z comprises a reporter moiety M wherein the reporter moiety comprises metal radionuclides, paramagnetic metal ions, fluorescent metal ions, heavy metal ions or cluster ions.

5. An agent as claimed in claim 5 wherein the reporter moiety M comprises ^{90}Y, ^{99m}Tc, ^{111}In, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{203}Pb, ^{141}Ce or ^{18}F.

6. An agent as claimed in any of the previous claims 1 to 5 where each reporter (Z) can carry a multiplicity of vectors V.

7. An agent as claimed in claims 1 and 2 where the antineoplastic agent . Z represent cyclophosphamide, chloroambucil, busulphan, methotrexate, cytarabine, fluorouracil, vinblastine, paclitaxel, doxorubicin, daunorubicin, etoposide, teniposide, cisplatin, amsacrine or docetaxel.

8. A peptide comprising the amino acid sequence of formula (II)

Z^1-Arg-Val(Arg/Lys)Ile-Asp-Gly-X^7-Pro-X^9-Z^2-Y^1 (II)

wherein

X^7 is an amino acid selected from the group Ala, Met, Gln, Arg, Glu and Val,
X^9 is an amino acid selected from the group Ala, Met, Gln, Arg, Gly and Val
Z^1 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue, or a residue capable of forming a thioether preferably the residue is Q-C(=O) wherein Q represents $-(CH_2)n$ or $-(CH_2)_n-C_6H_4$ where n represents a positive integer 1 to 10 or is absent and
Z^2 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue or is absent
Y^1 represents 1-10 amino acids or is absent
or pharmaceutically acceptable salts thereof.

9. A peptide comprising the amino acid sequence
Cys-Arg-Val-Arg-Ile-Asp-Gly-Ala-Pro-Ala-Cys, (SEQ ID NO 1),
Cys-Arg-Val-Arg-Ile-Asp-Asn-Met-Pro-Met-Cys, (SEQ ID NO 2),
Cys-Arg-Val-Arg-Ile-Asn-Gly-Gln-Pro-Gln-Cys, (SEQ ID NO 3),
Cys-Arg-Val-Lys-Ile-Asp-Gly-Arg-Pro-Met-Cys, (SEQ ID NO 4),
Cys-Arg-Leu-Lys-Ile-Asp-Gly-Met-Pro-Arg-Cys, (SEQ ID NO 5),
Cys-Arg-Ile-Lys-Ile-Asp-Gly-Glu-Gly-Gln-Cys, (SEQ ID NO 6),
Cys-Arg-Val-Tyr-Ile-Asp-Gly-Val-Ser-Val-Cys, (SEQ ID NO 7),
Cys-Arg-Val-Ile-Ile-Asp-Gly-Arg-Arg-Met-Cys, (SEQ ID NO 8),
Cys-Arg-Tyr-Asn-Ile-Asp-Gly-Arg-Pro-Gln-Cys, (SEQ ID NO 9) or
Cys-Arg-Ile-Arg-Ile-Asp-Gln-Arg-Pro-Ala-Cys, (SEQ ID NO 10).

10. A pharmaceutical composition comprising an effective amount of a compound of general Formula (III) or a salt thereof, together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

11. A method of generating enhanced images of a human or animal body previously administered with a contrast agent composition comprising a compound as claimed in claims 1 to 6, which method comprises generating an image of at least part of said body.

Lys - Lysine
Asn - Aspargine
Gln - Glutamine
Ala - Alanine
Met - Methionine
Glu - Glutamic acid

In a first aspect, the present invention provides a new peptide that targets VEGFR 2.

The new peptide comprising the amino acid sequence of formula (I)
Z^1-Arg-X^2-X^3-Ile-X^5-X^6-X^7-X^8-X^9-Z^2-Y^1 (Formula I)
wherein
X^2 is an amino acid selected from the group Val, Leu, Ile and Tyr
X^3 is an amino acid selected from the group Arg, Lys, Tyr, Ile and Asn
X^5 is an amino acid selected from the group Asp and Asn
X^6 is an amino acid selected from the group Gly, Asn and Gln
X^7 is an amino acid selected from the group Ala, Met, Gln, Arg, Glu and Val,
X^8 is an amino acid selected from the group Pro, Gly, Ser and Arg
X^9 is an amino acid selected from the group Ala, Met, Gln, Arg, Gly and Val

Z^1 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue, or a residue capable of forming a thioether preferably the residue is Q-C(=O) wherein Q represents $-(CH_2)n$ or $-(CH_2)_n-C_6H_4$ where n represents a positive integer 1 to 10 or is absent and
Z^2 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue or is absent
Y^1 represents 1-10 amino acids or is absent
or pharmaceutically acceptable salts thereof.

More specific the new peptide comprises the amino acid sequence of formula (II)
Z^1-Arg-Val(Arg/Lys)Ile-Asp-Gly-X^7-Pro-X^9-Z^2-Y^1 Formula (II)
wherein
X^7 is an amino acid selected from the group Ala, Met, Gln, Arg, Glu and Val,
X^9 is an amino acid selected from the group Ala, Met, Gln, Arg, Gly and Val
Z^1 represent an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue, or a residue capable of forming a thioether preferably the residue is Q-C(=O) wherein Q represents $-(CH_2)n$ or $-(CH_2)_n-C_6H_4$